

## THE EFFECT OF CHRONIC PROPRANOLOL TREATMENT ON OVERNIGHT PLASMA LEVELS OF ANTERIOR PITUITARY AND RELATED HORMONES

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- 1 Treatment of eight healthy males with propranolol (80 mg twice daily) for 6 weeks resulted in a significant reduction in overnight plasma levels of prolactin and LH.
- 2 Plasma testosterone levels were elevated whilst GH and cortisol levels were unchanged by such treatment.
- 3 Measurement of overnight hormone levels 48 h after discontinuing treatment showed no evidence of a 'rebound' phenomenon.
- 4 Cortisol, GH, prolactin, and testosterone plasma levels all showed time dependent changes: propranolol treatment significantly altered the time course of cortisol but not of the other hormones.
- 5 The effects of chronic propranolol treatment are discussed in terms of a probable direct central action of the drug. In addition the lowered plasma prolactin levels may directly contribute to the hypotensive action of propranolol.

### Introduction

$\beta$ -adrenoceptor blocking drugs are known to be effective in the treatment of hypertension although the mechanism by which they lower blood pressure has not been fully explained: both central (Lewis & Heusler, 1975) and peripheral (Conolly, Kersting & Dollery, 1976) mechanisms have been suggested. A recent study (Lewis *et al.*, 1981) of the effect of propranolol and acebutolol on overnight plasma levels of anterior pituitary, and related hormones provided further evidence for a central effect of these drugs. Thus single evening doses of propranolol (80 mg) and acebutolol (200 mg) were found to lower significantly the overnight plasma levels of follicle stimulating hormone (FSH) in a group of healthy male volunteers. Plasma prolactin levels were also reduced though the reduction did not reach significance with propranolol. Propranolol also elevated plasma cortisol levels and reduced plasma testosterone levels but it was suggested that these changes might be secondary to the acute haemodynamic effects peculiar to propranolol.

In addition to providing further support for a central effect of propranolol and acebutolol (both

lipid soluble  $\beta$ -adrenoceptor blocking drugs) the demonstration of a fall in plasma prolactin levels might be relevant to a hypotensive effect. Thus it is known that prolactin causes renal salt and water retention (Horrobin *et al.*, 1971), enhances the vasoconstrictor response to noradrenaline (Horrobin, Manku & Burstyn, 1973) and can increase cellular levels of sodium (Gopalakrishnan *et al.*, 1980). A fall in plasma prolactin levels might therefore contribute to the hypotensive effect of centrally active  $\beta$ -adrenoceptor blocking drugs.

The previous study provided no information on the effect of chronically administered  $\beta$ -adrenoceptor blocking drugs on plasma hormone levels. There are known to be marked differences in the haemodynamic changes following acute, as opposed to chronic, administration of  $\beta$ -adrenoceptor blockers (Hansson, 1979) and an extrapolation of results obtained following single dose  $\beta$ -adrenoceptor blocker treatment to a situation, such as the treatment of hypertension, involving chronic administration may not be warranted. Chronic treatment with the  $\beta$ -adrenoceptor-blocker tolamolol has previously been reported to increase plasma prolactin levels in female but not in male hypertensive subjects (Davies *et al.*, 1976). Tolamolol is not available for routine use. We studied the effects of chronic (6 weeks) therapy with propranolol on the

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overnight plasma levels of anterior pituitary and related hormones in normal men.

## Methods

Eight healthy male medical students (age 22–23 years) were studied. Informed consent was obtained from each subject after approval from the local ethical committee had been received. The subjects were studied on four separate occasions: pre-treatment, while taking propranolol 80 mg twice daily (after 6 weeks of such treatment), 48 h after discontinuing treatment, and 6 weeks after discontinuing treatment. On each occasion indwelling venous cannulae were inserted 2 h before taking the first blood sample and were kept patent by the slow infusion of 250 ml of heparinised saline over the 12 h sampling period. Blood sampling began at 21.00 h after all subjects had been recumbent for at least 30 min. Hourly 5 ml blood samples were taken between 21.00 h and 09.00 h the following morning, the venous line being emptied of heparinised saline before each sample was taken. The 5 ml blood sample was added to a tube containing ethylene-diamine-tetracetic acid potassium, the plasma being separated within 30 min and stored at  $-18^{\circ}\text{C}$  until assayed for prolactin, growth hormone (GH), luteinizing hormone (LH), cortisol and testosterone. All hormones were measured by radioimmunoassay, (Groom, 1977; Dyas, Read & Riad-Fahmy, 1979; Riad-Fahmy *et al.*, 1979).

Three way analysis of variance was used for statistical analysis of results. The 'main effects' of subject, time, and treatment status (pre-treatment, on treatment, 48 h post-treatment and 6 weeks post-treatment) were analysed. The two way interactions,

subject-treatment status, subject-time and time-treatment status, were also analysed. *F* values were calculated for each of the variables. Where a two way interaction was found to be significant the data were used to re-calculate the significance of the 'main effects' to give a more 'conservative' figure of the *F* values (for definition of statistical terms see Armitage, 1977).

## Results

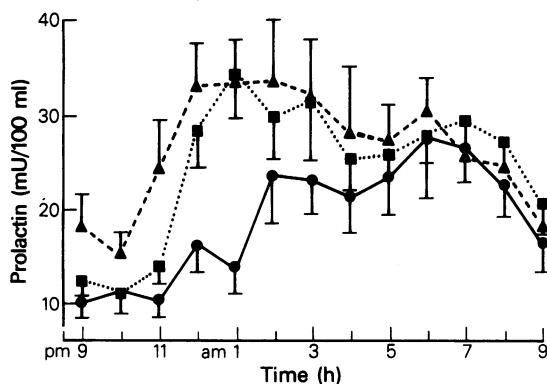
The 6 week post-treatment results were used as controls in view of the probable stress association with the initial overnight study as evidenced by the higher cortisol values at this time.

The overnight time course of plasma hormone values on treatment, 48 h post treatment and control (i.e. 6 weeks post-treatment) are shown in Figures 1, 2, 3, 4 and 5. Statistical analysis showed a significant ( $P < 0.01$ ) variation with time for all hormones except LH. There was also as might be expected a significant ( $P < 0.01$ ) inter-subject variability for all hormones. Analysis of the interaction between time and treatment showed that treatment did not affect the time course of any hormone level except cortisol (as described below). The results are summarised in Table 1 and the effects of chronic propranolol treatment are described in more detail for each hormone below.

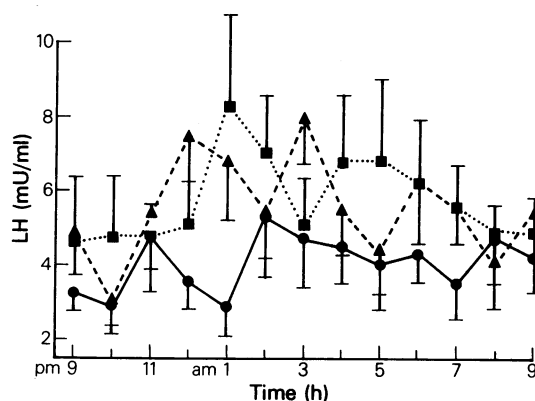
### Prolactin

Propranolol treatment significantly reduced the plasma levels of prolactin. There was no significant difference between the 48 h and 6 week post-treatment values.

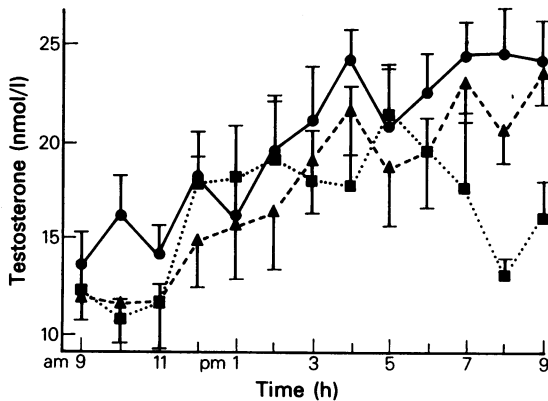
Analysis of the two way interactions showed no



**Figure 1** The overnight time course of plasma levels of prolactin during propranolol treatment (●), 48 h after propranolol withdrawal (▲) and 6 weeks after stopping propranolol treatment (■). Each point shows the mean  $\pm$  s.e. mean of eight subjects.



**Figure 2** The overnight time course of plasma levels of LH during propranolol treatment (●), 48 h after propranolol withdrawal (▲) and 6 weeks after stopping propranolol treatment (■). Each point shows the mean  $\pm$  s.e. mean of eight subjects.



**Figure 3** The overnight time course of plasma levels of testosterone during propranolol treatment (●), 48 h after propranolol withdrawal (▲) and 6 weeks after stopping propranolol treatment (■). Each point shows the mean  $\pm$  s.e. mean of eight subjects.

difference between subjects in their response to propranolol treatment or its withdrawal (at 48 h). Different subjects showed significant ( $P < 0.05$ ) variations in the time course irrespective of treatment status.

#### Luteinising hormone

Propranolol significantly reduced plasma levels of LH. There was no significant difference between the values 48 h and 6 weeks post treatment (Figure 2).

Analysis of two way interactions showed a significant inter-subject variability in response to propranolol treatment and its withdrawal (at 48 h), but no difference in the time course between different subjects.

#### Testosterone

Propranolol significantly elevated plasma testosterone levels. There was no significant difference between the 48 h and 6 weeks post treatment levels (Figure 3).

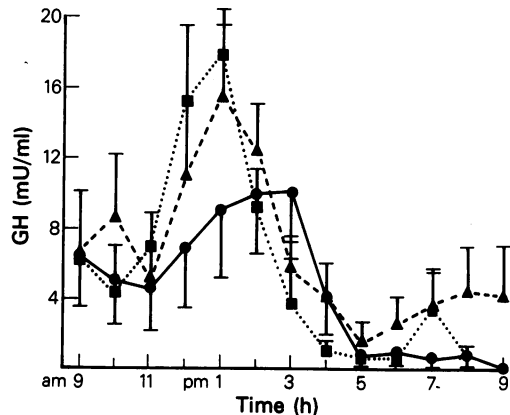
There was a significant inter-subject variability in the response to propranolol and its withdrawal (at 48 h), but no difference in time course between different subjects.

#### Growth hormone

Propranolol had no effect on GH levels and there were no significant two-way interactions (Figure 4).

#### Cortisol

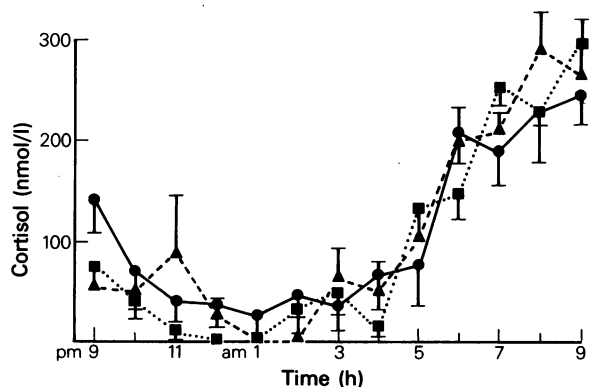
There was no effect of propranolol treatment or its withdrawal (at 48 h) on cortisol levels (Figure 5).



**Figure 4** The overnight time course of plasma levels of GH during propranolol treatment (●), 48 h after propranolol withdrawal (▲) and 6 weeks after stopping propranolol treatment (■). Each point shows the mean  $\pm$  s.e. mean of eight subjects.

Analysis of the two way interactions showed that there were significant differences between subjects in their response to propranolol and its withdrawal (at 48 h), and that the time course was also significantly different between different subjects.

Although there was no 'main effect' of propranolol treatment on plasma cortisol levels, there was an effect of such treatment on the time-related changes in plasma cortisol levels. Propranolol treatment elevated plasma cortisol levels in the first 6 h (21.00 h–03.00 h) of the overnight sampling period and reduced levels in the second 6 h period (03.00 h–09.00 h). Thus the diurnal variation in plasma cortisol levels was reduced by propranolol treatment.



**Figure 5** The overnight time course of plasma levels of cortisol during propranolol treatment (●), 48 h after propranolol withdrawal (▲) and 6 weeks after stopping propranolol treatment (■). Each point shows the mean  $\pm$  s.e. mean of eight subjects.

**Table 1** Statistical analysis (*P* values) of hormonal changes during and 48 h after stopping propranolol treatment compared with control levels (6 week post-propranolol).

<i>Main effects</i>	<i>Prolactin</i>	<i>LH</i>	<i>Testosterone</i>	<i>GH</i>	<i>Cortisol</i>
Subject	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Time	< 0.01	NS	< 0.01	< 0.01	< 0.01
Treatment status:					
on propranolol	< 0.01	< 0.01	< 0.01	NS	NS
48 h post-propranolol	NS	NS	NS	NS	NS
Two-way interactions					
Subject/Treatment:					
on propranolol	NS	< 0.01	< 0.01	NS	< 0.01
48 h post-propranolol	NS	< 0.01	< 0.01	NS	< 0.01
Subject/Time	< 0.05	NS	NS	NS	< 0.01
Time/Treatment:					
on propranolol	NS	NS	NS	NS	< 0.01
48 h post-propranolol	NS	NS	NS	NS	NS

## Discussion

The changes in plasma levels of prolactin, testosterone, GH and cortisol with time are in accordance with previous studies. Prolactin (Parker, Rossman & VanderLaan, 1973) and GH (Parker *et al.*, 1979) are both known to show sleep-related patterns of secretion. Cortisol secretion (Gallagher *et al.*, 1973) shows a diurnal rhythm. Testosterone (Judd & Parker, 1976) appears to have combined diurnal and sleep-related patterns of secretion. The absence of time-dependent changes in LH levels is also in agreement with previous studies (Yen *et al.*, 1974). There is reported evidence that prolactin (Parker *et al.*, 1973) and LH (Yen *et al.*, 1974) are secreted in 'pulses', but the sampling was too infrequent (1/h) in the present study to allow analysis in such terms. These time-dependent changes in plasma hormone levels throughout the night were not altered by propranolol, except in the case of cortisol.

The multivariate statistical analysis used in the present study showed also that there was a significant inter-subject variability in plasma levels for all hormones, consistent with the variation to be expected in biological systems.

The findings of this study on the effects of 6 weeks propranolol treatment differed in several respects from those of our previous similar study (Lewis *et al.*, 1981) on the effects of a single evening dose of propranolol. Prolonged propranolol treatment produced a significant reduction in the plasma levels of prolactin, whereas a single dose of propranolol caused only a small, and statistically insignificant, reduction. The reduction is therefore not related to an acute haemodynamic effect of the drug. It may contribute directly to the anti-hypertensive effect of propranolol. We have previously shown that bromocriptine reduces the raised levels of prolactin and further

lowers blood pressure in hypertensive subjects being treated with methyl dopa (Lewis & Henderson, 1980). On the other hand we showed no fall in prolactin levels in hypertensive patients treated chronically with oxprenolol, possibly because we used only single measurements to assess this dynamic hormonal system (Lewis *et al.*, 1979).

In contrast to the findings of the present study of the effects of propranolol in normal men, Davis *et al.* (1976) reported an elevation of plasma prolactin levels after chronic tolamolol treatment in female hypertensive subjects.

LH levels were significantly lowered by 6 weeks treatment with propranolol whereas testosterone levels rose. By contrast LH levels had been unchanged and testosterone and FSH levels lowered by the single dose of propranolol (Lewis *et al.*, 1981). Previous studies have shown no acute effect of propranolol on the pulses of LH release in man (Yen *et al.*, 1974) although in animals propranolol applied directly to the amygdala acutely increased plasma LH (Borrell *et al.*, 1977). The explanation for these differences is not clear. It is also unclear why there is a dissociation between the response of LH and testosterone to propranolol treatment. Previous studies have indicated that testosterone is secreted partly in response to pulses of LH with a time lag of approximately 1 h (Rubin *et al.*, 1975). However, there are clearly other factors involved in the secretion of testosterone since mean LH levels are not time dependent whereas testosterone levels are higher in the early morning than in the evening.

The changes produced by propranolol in plasma levels of prolactin testosterone and LH levels have been discussed in terms of alterations in secretion although it is possible that they could also arise from alterations in rates of metabolism. Little appears to be known about the intracellular fate of gonado-

trophins and prolactin or of any means of altering the rate of intracellular utilisation.

Growth hormone and cortisol levels were unaltered by propranolol treatment, suggesting that the changes previously seen after a single dose of propranolol might have been a response to acute haemodynamic changes. The effects of propranolol treatment on the time course of cortisol secretion is unexplained but could reflect a central effect of propranolol on the diurnal rhythm of ACTH release.

Comparison of the 48 h and 6 weeks post propranolol results with those obtained while on propranolol give no evidence for a 'rebound phenomenon' such as has been found with some other studies on propranolol withdrawal (Ross *et al.*, 1981).

The present study demonstrates an effect of chronic propranolol treatment on prolactin and gonadotrophin levels. These findings could at least in part be due to a direct central effect of propranolol. It is possible that the lower plasma prolactin levels might directly contribute to the anti-hypertensive effect of propranolol although such a possibility would have to be investigated in hypertensive subjects.

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